WO9603043

Publication Title:

HUMAN BRAIN SPECIFIC KINASE

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PCT WORLD INTELLECTUAL Internati

WO 9603043A1

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A01N 43/04, A61K 31/70, C07H 21/02, 21/04, C07K 1/00, 14/00, 16/00, 17/00, C12N 15/00, C12P 21/08, C12Q 1/68

A1

(11) International Publication Number: WO 96/03043

(43) International Publication Date: 8 February 1996 (08.02.96)

(21) International Application Number: PCT/US95/09334

(22) International Filing Date: 26 July 1995 (26.07.95)

(30) Priority Data:
08/279,855 26 July 1994 (26.07.94) US

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(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

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The present invention relates, in general, to human brain specific kinase, hBsk. In particular, the present invention relates to nucleic acid molecules coding for hBsk; hBsk polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antibodies having binding affinity specifically to hBsk; hybridomas containing the antibodies; nucleic acid probes for the detection of hBsk nucleic acid; a method of detecting hBsk nucleic acid or polypeptide in a sample; and kits containing nucleic acid probes or antibodies. This invention further relates to bioassays using the nucleic acid sequence, receptor protein or antibodies of this invention to diagnose, assess, or prognose a mammal afflicted with neurodegenerative disease. Therapeutic uses for the hBsk receptor-like tyrosine kinase are also provided. This invention also relates to the ligand for the hBsk receptor, and diagnostic and therapeutic uses for the hBsk ligand.

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HUMAN BRAIN SPECIFIC KINASE

Background of the Invention

Field of the Invention

The present invention relates, in general, to human brain specific kinase, hBsk. In particular, the present invention relates to nucleie acid molecules coding for hBsk; hBsk polypeptides; recombinant nucleic acid molecules; cells containing the recombinant nucleic acid molecules; antibodies having binding affinity specifically to hBsk; hybridomas containing the antibodies; nucleic acid probes for the detection of hBsk nucleic acid; a method of detecting hBsk nucleic acid or polypeptide in a sample; and kits containing nucleic acid probes or antibodies.

Background Information

Neuronal degeneration has been shown to be involved in many neurological disorders (Price *et al.*, in *Selective Neuronal Death*, Ciba Foundation Symposium 126, Wiley & Chichester, eds. (1987), pp. 30-48). For example, in Alzheimer's Disease (AD), neuronal loss has been reported in a variety of brain structures including the locus coeruleus and raphe complex of the brainstem, the basal forebrain cholinergic system, amygdala, hippocampus and neocortex (Coleman & Flood, *Neurobiol. Aging 8*:521-545 (1987)). Although the pattern of cell loss in AD has similarities to that in the aging brain, the speed and amount of loss is far greater. The most striking loss of neurons compared with the age-matched controls occurs in the hippocampal region, with a loss of up to 57% of the pyramidal cells (Coleman and Flood, *Neurobiol. Aging 8*:521-545 (1987)). These observation indicate that the hippocampus is a key structure in the neurobiology of AD. The extent of cell loss is most evident in the CA1

and subiculum, while areas CA3 and CA4 and granular cells of the dentate gyrus are largely spared (Van Hoesen and Hyman, *Progress in Brain Research 83*:445-457 (1990)).

Neuronal degeneration in the hippocampus has long been known to be a site of pathological change in epileptic patients (Nadler, in *The Hippocampus-New Vistas*, Chan-Palay V., Koehler C., eds.,, Alan R. Liss, Inc., New York (1989), pp. 463-481). CA3-CA4 damage is nearly always observed in pharmacologically intractable complex partial (limbic, temporal lobe, psychomotor) epileptic patients, while CA1 damage is also frequently observed (Nadler, in *The Hippocampus-New Vistas*, Chan-Palay V., Koehler C., eds.,, Alan R. Liss, Inc., New York (1989), pp. 463-481). Since frequently prominent cell loss in the CA3-CA4 area may be present without obvious damage to area CA1, it is believed that the CA3 pyramidal cells and the morphologically diverse CA4 neurons are most vulnerable. Although the relationship between hippocampal sclerosis and seizure has been a controversy for over a century, recent studies with animal models indicate that although other etiologies may exist, hippocampal lesions can arise from prolonged febrile convulsions/status epilepticus (Sutula, *Epilepsia 31*:345-554 (1990)). Furthermore, whatever the original cause of the sclerotic lesion, the damage serves as a focus for hyperexcitability and eventually causes spontaneous seizures. Formation of aberrant excitatory circuitry through axon sprouting and permanently depressed synaptic inhibition were thought to be two major factors linking hippocampal damage to the subsequent development of an epileptic focus (Nadler, in *The Hippocampus-New Vistas*, Chan-Palay V., Koehler C., eds.,, Alan R. Liss, Inc., New York (1989), pp. 463-481; Sutula, *Epilepsia 31* (Suppl. 3):545-554 (1990)).

Hippocampal defects are also suggested to be involved in schizophrenia (Bogerts *et al.*, *Arch. Gen. Psychiatry 42*:784-791 (1985)). Significant reductions in hippocampal volume were found in chronic

schizophrenic patients, possibly due to degenerative shrinkages of unknown etiology (Bogerts *et al.*, *Arch. Gen. Psychiatry 42*:784-791 (1985); Bogerts *et al.*, *Biol. Psychiatry 33*:236-246 (1993). The reduced volume in hippocampus and other limbic system structures such as amygdala and parahippocampal gyrus was associated with increased severity of psychopathology (Bogerts *et al.*, *Biol. Psychiatry 33*:236-246 (1993)). These changes in the limbic system in schizophrenia are rather specific since the volumes of the putamen, caudate nucleus, nucleus accumbens, and the red nucleus of the stria terminalis did not differ between patients and controls (Bogerts *et al.*, *Biol. Psychiatry 33*:236-246 (1993)).

The hippocampus and its adjacent, anatomically related entorhinal, perirhinal, and parahippocampal cortices play an essential, although temporal, role for establishing long-term memory for facts and events (Squire and Zola-Morgan, *Science 253:*1380-1386 (1991)). The widespread and reciprocal connections between hippocampal structures and neocortex may explain their degeneration in a variety of neurological diseases. Understanding the mechanism of neuronal survival in the hippocampus may help to develop effective treatments of neural degenerative diseases or disorders as well as neoplasms involving neuronal tissue.

It is known that growth/trophic factors promote the differentiation and survival of neurons during development and regeneration of the nervous system, with specific types of neurons requiring specific growth factors (Barde, *Neuron 2*:1525-1534 (1989)). Nerve growth factor (NGF) has been a model trophic factor (Levi-Montalcini, *Science 237*:1154-1162 (1987); Black *et al.*, in *Current Topics in Developmental Biology*, Volume 24, Academic Press Inc., (1990), pp. 161-182; Gage *et al.*, *Current Topics in Microbiology and Immunology*, Volume 165, Bothwell, M., ed., Springer Verlag, (1991), pp. 71-92). *In vivo* depletion by inducing an auto-immune reaction to NGF in rats and guinea pigs during embryonic development results in the destruction of up to 85% of the

dorsal root ganglion neurons and the destruction of peripheral sympathetic neurons (Colkart-Gorin an Johnson, *Proc. Natl. Acad. Sci. USA 76*:5382-5386 (1979); Johnson *et al.*, *Science 210*:916-918 (1980)). Other polypeptide growth factors also have trophic effects on neurons (Nieto-Sampedro and Vovolenta, in *Progress in Brain Research*, Vol. 83, Mathison, Zimmer, Otterson, eds., Elsevier, New York (1990), pp. 341-355). The fibroblast growth factors are well known mitogens (Gospodarowicz, in *Current Topics in Developmental Biology*, Vol. 24, Hilsen-Hamilton, M. ed., Academic Press Inc., San Diego (1990), pp. 57-93) that exhibit potent neurotrophic activity both *in vivo* (Anderson *et al.*, *Nature 332*:360-361 (1988)) and on cultured neurons from many brain regions (Morrison *et al.*, *Proc. Natl. Acad. Sci. USA 83*:7537-7541 (1986); Morrison, *Neuroscience Res. 17*:99-101 (1987); Walicke *J. Neursci. 6*:1114-1121 (1988); Wagner, in *Current Topics in Microbiology and Immunology*, Vol. 165, Bothwell, M. ed., Springer Verlag, New York (1991), pp. 95-112) including the hippocampus (Walicke *et al.*, *Proc. Natl. Acad. Sci. USA 83*:3012-3016 (1986). Epidermal growth factor (EFG) was shown to enhance the survival and process outgrowth of primary cultures of subneocortical telencephalic neurons of neonatal rat brain (Morrison *et al.*, *Science 238*:72-75 (1987)). Brain derived neurotrophic factor (BDNF) (Leibrock *et al.*, *Nature 341*:149-152 (1989), and neurotrophin-3 (NT-3) (Masisonpierre *et al.*, *Science 247*:1446-1451 (1990); Hohn *et al.*, *Nature 344*:339-341 (1990)) are two neurotrophic factors cloned recently. BDNF, related to NGF, has neurotrophic activity for sensory and retinal ganglion neurons and rescues spinal motor neurons *in vivo* from axotomy-induced cell death (Sendtner *et al.*, *Nature 360* (1992); Yan *et al.*, *Nature 360*:753-755 (1992)). NT-3 was shown to support the growth of neurons from dorsal root ganglion, the neural placode-derived nodose ganglion, and the paravertebral chain sympathetic ganglion (Maisonpierre *et al.*, *Science 247*:1446-1451 (1990)).

The potential involvement of growth factors in neuronal regeneration after injuries or in disease is demonstrated by the fact that brain injury causes a time dependent increase in neurotrophic activity at the lesion site (Nieto-Sampedro *et al.*, *Science 217*:860-861 (1982). Furthermore, intraventricular administration of NGF prevents retrograde degeneration of axotomized septal cholinergic neurons (Hefti *J. Neurosci. 6*:2155-2162 (1986); Kromer, *Science 235*:214-216 (1987); Williams *et al.*, *Proc. Natl. Acad. Sci. USA 83*:9231-9235 (1986)) and local application of BDNF prevents spinal motor neuron degeneration after nerve section (Sendtner *et al.*, *Nature 360*:753-755 (1992)). The therapeutic value of neurotrophic factors for nerve injury or neurodegenerative disease is shown by the observation that the symptoms of he mice with progressive motor neuropathy is relieved by the use of ciliary neurotrophic factor (Sendtner *et al.*, *Nature 258*:502-504 (1992)).

Growth/trophic factors function through their receptors which often possess intrinsic protein tyrosine kinase activity (Schlessinger & Ullrich, *Neuron 9*:383-931 (1992)). In general, the receptor protein-tyrosine kinases are composed of an extracellular domain, a membrane spanning domain and a catalytic domain (Schlessinger and Ullrich, *Neuron 9*:383-391 (1992). Binding of the growth/trophic factor to the extracellular domain activates the catalytic domain inside the cell and results in phosphorylation of substrates within the cell. Activation of the receptor is believed to mediate a variety of cellular processes including cell growth and differentiation. In addition, many receptor tyrosine kinases are expressed during embryogenesis and are therefore believed to be important in the mechanisms underlying oncogenesis and cellular growth (Wilks, *Advances in Cancer Research 60*:43-73 (1993)). Increased or aberrant expressions of tyrosine kinase receptors has been associated with several human neoplasms, including glioblastomas, squamous carcinomas, breast and gastric cancers (Carpenter, *Ann. Rev. Biochem. 56*: 881-914 (1987); Muller

et al., *Cell 54*:105-109 (1989); Kraus *et al.*, *Proc. Natl. Acad. Sci. USA 86*:9193-9197 (1989)). Recently, it has been demonstrated that expression of *trk*, the tyrosine kinase receptor for NGF, in neuroblastomas is indicative of a better prognosis for the patient than those patients having neuroblastomas without *trk* expression (Kogner, *et al.*, *Cancer Research 53*:2044-2050 (1993); Nakagawara *et al.*, *New Eng. J. Med. 328*:847-854 (1993); Suzuki *et al.*, *J. Natl. Can. Inst. 85*:377-384 (1993)), suggesting a therapeutic value for the neurotrophic receptors as well as their ligands.

Recently, a new family of tyrosine kinase receptors have been discovered and designated the *eph/elk* family (Zhou *et al.*, *J. Neuroscience Re. 37(1)*:129-143 (1994); Sajjadi *et al.*, *Oncogene 8*:1807-1813 (1993)). Members of the *eph/elk* family have also been demonstrated to have aberrant expression in certain neoplasm as well as transforming ability. For example, elevated expression of *EPH* has been detected in carcinomas of the liver, lung, breast and colon (Hirai *et al.*, *Science 238*:1717-1720 (1987); Maru *et al.*, *Mol. Cell Biol. 8*:3770-3776 (1988)). Overexpression of *eph* has been shown to result in transformation of cells as well as development of tumors in nude mice (Maru *et al.*, *Oncogene 5*:445-447 (1990)). The distinct tissue distributions of the *eph/elk* family members suggest that each member may serve specific functions.

These findings demonstrate the extensive involvement of growth factors and their corresponding receptors in the survival of neurons and their potential therapeutic value in neurodegenerative diseases, neuronal disorders and neoplasms. Alzheimer's epilepsy and schizophrenia are but a few of the diseases associated with degeneration of neurons in the hippocampus. However, the factors needed for the regeneration and survival of neurons in the hippocampus and its associated limbic system are poorly characterized. The identification of factors which promote the regeneration and survival of these neurons will be potentially useful in the

treatment of the neoplasms, neurodegenerative diseases or disorders and brain injuries involving the limbic system.

Zhou *et al., J. of Neuroscience Res. 37:*129-143 (1994) describes the isolation and characterization of a mouse Bsk.

Summary of the Invention

The invention provides an isolated nucleic acid molecule coding for a polypeptide comprising an amino acid sequence corresponding to human brain specific kinase, hBsk.

The invention further provides a substantially pure polypeptide comprising an amino acid sequence corresponding to hBsk.

The invention also provides a nucleic acid probe for the specific detection of the presence of hBsk in a sample.

The invention further provides a method of detecting hBsk nucleic acid in a sample.

The invention also provides a kit for detecting the presence of hBsk nucleic acid in a sample.

The invention further provides a recombinant nucleic acid molecule comprising, 5′ to 3′, a promoter effective to initiate transcription in a host cell and the above-described isolated nucleic acid molecule.

The invention also provides a recombinant nucleic acid molecule comprising a vector and the above-described isolated nucleic acid molecule.

The invention further provides a recombinant nucleic acid molecule comprising a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide.

The invention also provides a cell that contains the above-described recombinant nucleic acid molecule.

The invention further provides a non-human organism that contains the above-described recombinant nucleic acid molecule.

The invention also provides an antibody having binding affinity specifically to a hBsk polypeptide.

The invention further provides a method of detecting a hBsk polypeptide in a sample.

The invention also provides a method of measuring the amount of hBsk in a sample.

The invention further provides a diagnostic kit comprising a first container means containing the above-described antibody, and a second container means containing a conjugate comprising a binding partner of said monoclonal antibody and a label.

The invention also provides a hybridoma which produces the above-described monoclonal antibody.

The invention further provides diagnostic methods for human disease, in particular neurodegenerative diseases, disorders, and injuries.

The invention also provides methods for therapeutic uses involving all or part of the nucleic acid sequence encoding hBsk and its corresponding protein.

The invention provides assays for the isolation of the ligand or ligands capable of activating the hBsk receptor and therapeutic uses for said ligand.

The invention also provides assays for the assessment and development of drugs capable of activating the hBsk receptor and therapeutic uses for said drugs.

Further objects and advantages of the present invention will be clear from the description that follows.

Brief Description of the Drawings

FIGURE 1. Schematic of overlapping human Bsk cDNA Isolates. Human Bsk cDNA clones are shown with their extent of overlap under a

schematic of the mouse Bsk gene comprising: an extracellular domain (E), transmembrane domain (TM) and a kinase domain (K). The nucleotide numbers at the 3' and/or 5' ends of the isolates represent the corresponding nucleotide number within the mouse Bsk sequence (*See*, Zhou *et al.* (1994) *J. Neuroscience Res. 37:*129-143.

FIGURE 2A-2J. Partial Nucleotide Sequence of a hBsk gene. Figure 2A, 2C, 2E, 2G, and 2I describe a partial nucleotide sequence of Clones 6-1, 7-2, 8-1, 16-1, and 19-1, respectively, using an sk primer. Figure 2B, 2D, 2F, 2H, and 2J describe a partial nucleotide sequence of Clones 6-1, 7-2, 8-1, 16-1, and 19-1, respectively, using a T7 primer.

Definitions

In the description that follows, a number of terms used in recombinant DNA (rDNA) technology are extensively utilized. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

Isolated Nucleic Acid Molecule. An "isolated nucleic acid molecule", as is generally understood and used herein, refers to a polymer of nucleotides, and includes but should not be limited to DNA and RNA.

DNA Segment. A DNA segment, as is generally understood and used herein, refers to a molecule comprising a linear stretch of nucleotides wherein the nucleotides are present in a sequence that may encode, through the genetic code, a molecule comprising a linear sequence of amino acid residues that is referred to as a protein, a protein fragment or a polypeptide.

Gene. A DNA sequence related to a single polypeptide chain or protein, and as used herein includes the 5' and 3' untranslated ends. The

polypeptide can be encoded by a full-length sequence or any portion of the coding sequence, so long as the functional activity of the protein is retained.

Complementary DNA (cDNA). Recombinant nucleic acid molecules synthesized by reverse transcription of messenger RNA ("mRNA").

Structural Gene. A DNA sequence that is transcribed into mRNA that is then translated into a sequence of amino acids characteristic of a specific polypeptide.

Restriction Endonuclease. A restriction endonuclease (also restriction enzyme) is an enzyme that has the capacity to recognize a specific base sequence (usually 4, 5, or 6 base pairs in length) in a DNA molecule, and to cleave the DNA molecule at every place where this sequence appears. For example, *Eco*RI recognizes the base sequence GAATTC/CTTAAG.

Restriction Fragment. The DNA molecules produced by digestion with a restriction endonuclease are referred to as restriction fragments. Any given genome may be digested by a particular restriction endonuclease into a discrete set of restriction fragments.

Agarose Gel Electrophoresis. To detect a polymorphism in the length of restriction fragments, an analytical method for fractionating double-stranded DNA molecules on the basis of size is required. The most commonly used technique (though not the only one) for achieving such a fractionation is agarose gel electrophoresis. The principle of this method is that DNA molecules migrate through the gel as though it were a sieve that retards the movement of the largest molecules to the greatest extent and the movement of the smallest molecules to the least extent. Note that the smaller the DNA fragment, the greater the mobility under electrophoresis in the agarose gel.

The DNA fragments fractionated by agarose gel electrophoresis can be visualized directly by a staining procedure if the number of fragments

included in the pattern is small. The DNA fragments of genomes can be visualized successfully. However, most genomes, including the human genome, contain far too many DNA sequences to produce a simple pattern of restriction fragments. For example, the human genome is digested into approximately 1,000,000 different DNA fragments by *Eco*RI. In order to visualize a small subset of these fragments, a methodology referred to as the Southern hybridization procedure can be applied.

Southern Transfer Procedure. The purpose of the Southern transfer procedure (also referred to as blotting) is to physically transfer DNA fractionated by agarose gel electrophoresis onto a nitrocellulose filter paper or another appropriate surface or method, while retaining the relative positions of DNA fragments resulting from the fractionation procedure. The methodology used to accomplish the transfer from agarose gel to nitrocellulose involves drawing the DNA from the gel into the nitrocellulose paper by capillary action.

Nucleic Acid Hybridization. Nucleic acid hybridization depends on the principle that two single-stranded nucleic acid molecules that have complementary base sequences will reform the thermodynamically favored double-stranded structure if they are mixed under the proper conditions. The double-stranded structure will be formed between two complementary single-stranded nucleic acids even if one is immobilized on a nitrocellulose filter. In the Southern hybridization procedure, the latter situation occurs. As noted previously, the DNA of the individual to be tested is digested with a restriction endonuclease, fractionated by agarose gel electrophoresis, converted to the single-stranded form, and transferred to nitrocellulose paper, making it available for reannealing to the hybridization probe.

Hybridization Probe. To visualize a particular DNA sequence in the Southern hybridization procedure, a labeled DNA molecule or hybridization probe is reacted to the fractionated DNA bound to the nitrocellulose filter. The areas on the filter that carry DNA sequences complementary to the

labeled DNA probe become labeled themselves as a consequence of the reannealing reaction. The areas of the filter that exhibit such labeling are visualized. The hybridization probe is generally produced by molecular cloning of a specific DNA sequence.

Oligonucleotide or Oligomer. A molecule comprised of two or more deoxyribonucleotides or ribonucleotides, preferably more than three. Its exact size will depend on many factors, which in turn depend on the ultimate function or use of the oligonucleotide. An oligonucleotide may be derived synthetically or by cloning.

Sequence Amplification. A method for generating large amounts of a target sequence. In general, one or more amplification primers are annealed to a nucleic acid sequence. Using appropriate enzymes, sequences found adjacent to, or in between the primers are amplified.

Amplification Primer. An oligonucleotide which is capable of annealing adjacent to a target sequence and serving as an initiation point for DNA synthesis when placed under conditions in which synthesis of a primer extension product which is complementary to a nucleic acid strand is initiated.

Vector. A plasmid or phage DNA or other DNA sequence into which DNA may be inserted to be cloned. The vector may replicate autonomously in a host cell, and may be further characterized by one or a small number of endonuclease recognition sites at which such DNA sequences may be cut in a determinable fashion and into which DNA may be inserted. The vector may further contain a marker suitable for use in the identification of cells transformed with the vector. Markers, for example, are tetracycline resistance or ampicillin resistance. The words "cloning vehicle" are sometimes used for "vector."

Expression. Expression is the process by which a structural gene produces a polypeptide. It involves transcription of the gene into mRNA, and the translation of such mRNA into polypeptide(s).

Expression Vector. A vector or vehicle similar to a cloning vector but which is capable of expressing a gene which has been cloned into it, after transformation into a host. The cloned gene is usually placed under the control of (i.e., operably linked to) certain control sequences such as promoter sequences.

Expression control sequences will vary depending on whether the vector is designed to express the operably linked gene in a prokaryotic or eukaryotic host and may additionally contain transcriptional elements such as enhancer elements, termination sequences, tissue-specificity elements, and/or translational initiation and termination sites.

Functional Derivative. A "functional derivative" of a sequence, either protein or nucleic acid, is a molecule that possesses a biological activity (either functional or structural) that is substantially similar to a biological activity of the protein or nucleic acid sequence. A functional derivative of a protein may or may not contain post-translational modifications such as covalently linked carbohydrate, depending on the necessity of such modifications for the performance of a specific function. The term "functional derivative" is intended to include the "fragments," "segments," "variants," "analogs," or "chemical derivatives" of a molecule.

As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, and the like. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, and the like. Moieties capable of mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980). Procedures for coupling such moieties to a molecule are well known in the art.

Variant. A "variant" of a protein or nucleic acid is meant to refer to a molecule substantially similar in structure and biological activity to

either the protein or nucleic acid. Thus, provided that two molecules possess a common activity and may substitute for each other, they are considered variants as that term is used herein even if the composition or secondary, tertiary, or quaternary structure of one of the molecules is not identical to that found in the other, or if the amino acid or nucleotide sequence is not identical.

Substantially Pure. A "substantially pure" protein or nucleic acid is a protein or nucleic acid preparation that is generally lacking in other cellular components.

Ligand. Ligand refers to any protein or proteins that may interact with the hBsk receptor binding domain. Said ligand or ligands may be soluble or membrane bound. The ligand or ligands may be a naturally occurring protein, or synthetically or recombinantly produced. The ligand may also be a nonprotein molecule that acts as ligand when it interacts with the Bsk receptor binding domain. Interactions between the ligand and receptor binding domain include, but are not limited to, any covalent or non-covalent interactions. The receptor binding domain is any region of the hBsk receptor molecule that interacts directly or indirectly with the hBsk ligand.

Neurodegenerative disease. The term neurodegenerative disease includes, but is not limited to, states in a mammal which can include chromosomal abnormalities, degenerative growth and developmental disorders, viral infections, bacterial infections, brain injuries, or neoplastic conditions. Examples of neurodegenerative diseases that can be diagnosed, assessed or treated by methods described in the present application include, but are not limited to, Alzheimer's disease, epilepsy, schizophrenia. In a preferred embodiment diseases characterized by neurodegeneration in the limbic system are diagnosed, assessed or treated by methods disclosed in the present application. Examples of injuries to the nervous system include, but are not limited to, stroke and cerebral ischemia due to stroke

or cardiac arrest. Also considered within this definition is the treatment of injury to the nervous system. Further, neoplasms involving neuronal tissue may be diagnosed, assessed or therapeutically treated by methods suggested herein.

Drug. Drugs include, but are not limited to proteins, peptides, degenerate peptides, agents purified form conditioned cell medium, organic molecules, inorganic molecules, antibodies or oligonucleotides. Other candidate drugs include analogs of the hBsk ligand or ligands. The drug may be naturally occurring or synthetically or recombinantly produced. One skilled in the art will understand that such drugs may be developed by the assays described below.

Detailed Description of the Invention

For purposes of clarity of disclosure, and not by way of limitation, the detailed description of the invention is divided into the following subsections:

I. Isolated Nucleic Acid Molecules Coding for hBsk Polypeptides.

II. Substantially Pure hBsk Polypeptides.

III. A Nucleic Acid Probe for the Specific Detection of hBsk.

IV. A Method of Detecting The Presence of hBsk in a Sample.

V. A Kit for Detecting the Presence of hBsk in a Sample.

VI. DNA Constructs Comprising a hBsk Nucleic Acid Molecule and Cells Containing These Constructs.

VII. An Antibody Having Binding Affinity to an hBsk Polypeptide and a Hybridoma Containing the Antibody.

VIII. A Method of Detecting an hBsk Polypeptide in a Sample.

IX. A Diagnostic Kit Comprising Antibodies to hBsk.

X. Diagnostic Screening and Treatment

I. Isolated Nucleic Acid Molecules Coding for hBsk Polypeptides

In one embodiment, the present invention relates to an isolated nucleic acid molecule coding for a polypeptide having an amino acid sequence corresponding to human brain specific kinase, hBsk. In one preferred embodiment, the isolated nucleic acid molecule comprises the hBsk nucleotide sequence present in Clones 6-1 and 8-1 (See Figure 1) as deposited with the ATCC. In another preferred embodiment, the isolated nucleic acid molecule encodes the hBsk amino acid sequence present in Clones 6-1 and 8-1 (See Figure 1) as deposited with the ATCC. The hBsk sequence within Clones 6-1 and 8-1 is identified by its homology to the mouse bsk sequence (See Figure 1).

Due to the degeneracy of nucleotide coding sequences, other DNA sequences which encode the same amino acid sequence as found in Clones 6-1 and 8-1 may be used in the practice of the present invention.

A. Isolation of Nucleic Acid

In one aspect of the present invention, isolated nucleic acid molecules coding for polypeptides having amino acid sequences corresponding to hBsk are provided. In particular, the nucleic acid molecule may be isolated from a biological sample containing human RNA or DNA.

The nucleic acid molecule may be isolated from a biological sample containing human RNA using the techniques of cDNA cloning and subtractive hybridization. The nucleic acid molecule may also be isolated from a cDNA library using a homologous probe.

The nucleic acid molecule may be isolated from a biological sample containing human genomic DNA or from a genomic library. Suitable biological samples include, but are not limited to, blood, semen and tissue. The method of obtaining the biological sample will vary depending upon the nature of the sample.

One skilled in the art will realize that the human genome may be subject to slight allelic variations between individuals. Therefore, the isolated nucleic acid molecule is also intended to include allelic variations, so long as the sequence is a functional derivative of the hBsk gene. When a hBsk allele does not encode the identical sequence to that found in Clones 6-1 and 8-1, it can be isolated and identified as hBsk using the same techniques used herein, and especially PCR techniques to amplify the appropriate gene with primers based on the sequences disclosed herein.

B. Synthesis of Nucleic Acid

Isolated nucleic acid molecules of the present invention are also meant to include those chemically synthesized. For example, a nucleic acid molecule with the nucleotide sequence which codes for the expression product of a hBsk gene may be designed and, if necessary, divided into appropriate smaller fragment. Then an oligomer which corresponds to the nucleic acid molecule, or to each of the divided fragments, may be synthesized. Such synthetic oligonucleotides may be prepared, for example, by the triester method of Matteucci *et al.*, *J. Am. Chem. Soc.* *103*:3185-3191 (1981) or by using an automated DNA synthesizer.

An oligonucleotide may be derived synthetically or by cloning. If necessary, the 5′-ends of the oligomers may be phosphorylated using T4 polynucleotide kinase. Kinasing of single strands prior to annealing or for labeling may be achieved using an excess of the enzyme. If kinasing is for the labeling of probe, the ATP may contain high specific activity

radioisotopes. Then, the DNA oligomer may be subjected to annealing and ligation with T4 ligase or the like.

II. Substantially Pure hBsk Polypeptides

In another embodiment, the present invention relates to a substantially pure polypeptide having an amino acid sequence corresponding to hBsk. In a preferred embodiment, the polypeptide has the amino acid sequence set forth in Clones 6-1 and 8-1 (See Figure 1).

A variety of methodologies known in the art can be utilized to obtain the peptide of the present invention. In one embodiment, the peptide is purified from human tissues or cells which naturally produce the peptide. Alternatively, the above-described isolated nucleic acid fragments could be used to expressed the hBsk protein in any organism. The samples of the present invention include cells, protein extracts or membrane extracts of cells, or biological fluids. The sample will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts used as the sample.

One skilled in the art can readily follow known methods for isolating proteins in order to obtain the peptide free of natural contaminants. These include, but are not limited to: immunochromotography, size-exclusion chromatography, HPLC, ion-exchange chromatography, and immunoaffinity chromatography.

III. A Nucleic Acid Probe for the Specific Detection of hBsk

In another embodiment, the present invention relates to a nucleic acid probe for the specific detection of the presence of hBsk in a sample comprising the above-described nucleic acid molecules or at least 18 contiguous nucleotides thereof (preferably at least 20, 25, 30, 35, 40, or 50

thereof). The probe is designed such that it does not have 100% homology with a similarly located mouse Bsk probe.

The nucleic acid probe may be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain another nucleic acid molecule of the present invention. A chromosomal DNA or cDNA library may be prepared from appropriate cells according to recognized methods in the art (cf. *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

In the alternative, chemical synthesis is carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to N-terminal and C-terminal portions of the amino acid sequence of the hBsk. Thus, the synthesized nucleic acid probes may be used as primers in a polymerase chain reaction (PCR) carried out in accordance with recognized PCR techniques, essentially according to *PCR Protocols, A Guide to Methods and Applications*, edited by Michael *et al.*, Academic Press, 1990, utilizing the appropriate chromosomal or cDNA library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes based on the sequence disclosed herein using methods of computer alignment and sequence analysis known in the art (cf. *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

The hybridization probes of the present invention can be labeled by standard labeling techniques such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and the like. After hybridization, the probes may be visualized using known methods.

The nucleic acid probes of the present invention include RNA, as well as DNA probes, such probes being generated using techniques known in the art.

In one embodiment of the above described method, a nucleic acid probe is immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, and acrylic resins, such as polyacrylamide and latex beads. Techniques for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The sample used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample which is compatible with the method utilized.

IV. A Method of Detecting The Presence of hBsk in a Sample

In another embodiment, the present invention relates to a method of detecting the presence of hBsk in a sample comprising a) contacting said sample with the above-described nucleic acid probe, under conditions such that hybridization occurs, and b) detecting the presence of said probe bound to said nucleic acid molecule. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples of human tissue.

hBsk has been found to be expressed in brain cells. Accordingly, hBsk probes may be used detect the presence of RNA from brain cells in a sample. Further, altered expression levels of hBsk RNA in an individual, as compared to normal levels, may indicate the presence of disease. The

hBsk probes may further be used to assay cellular activity in general and specifically in brain tissue.

V. A Kit for Detecting the Presence of hBsk in a Sample

In another embodiment, the present invention relates to a kit for detecting the presence of hBsk in a sample comprising at least one container means having disposed therein the above-described nucleic acid probe. In a preferred embodiment, the kit further comprises other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatic labeled probes (horse radish peroxidase, alkaline phosphatase), and affinity labeled probes (biotin, avidin, or steptavidin).

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like.

One skilled in the art will readily recognize that the nucleic acid probes described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

VI. DNA Constructs Comprising a hBsk Nucleic Acid Molecule and Cells Containing These Constructs

In another embodiment, the present invention relates to a recombinant DNA molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described nucleic acid molecules. In another embodiment, the present invention relates to a recombinant DNA molecule comprising a vector and an above-described nucleic acid molecule.

In another embodiment, the present invention relates to a nucleic acid molecule comprising a transcriptional control region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to the above-described polypeptide, and a transcriptional termination region functional in said cell.

Preferably, the above-described molecules are isolated and/or purified DNA molecules.

In another embodiment, the present invention relates to a cell or non-human organism that contains an above-described nucleic acid molecule.

In another embodiment, the peptide is purified from cells which have been altered to express the peptide.

As used herein, a cell is said to be "altered to express a desired peptide" when the cell, through genetic manipulation, is made to produce a protein which it normally does not produce or which the cell normally produces at low levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences

are "operably linked" to nucleotide sequences which encode the polypeptide. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression may vary from organism to organism, but shall in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the sequence encoding an hBsk gene may be obtained by the above-described methods. This region may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA sequence encoding a hBsk gene, the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted.

Two DNA sequences (such as a promoter region sequence and an hBsk sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region sequence to direct the transcription of a hBsk gene sequence, or (3) interfere with the ability of the hBsk gene sequence to be transcribed by the promoter region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence.

The present invention encompasses the expression of the hBsk gene (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, the most efficient and convenient for the production of recombinant proteins and, therefore, are preferred for the expression of the hBsk gene.

Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains may also be used, including other bacterial strains. In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host may be used. Examples of suitable plasmid vectors may include pBR322, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors may include λgt10, λgt11 and the like; and suitable virus vectors may include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.

Recognized prokaryotic hosts include bacteria such as *E. coli, Bacillus, Streptomyces, Pseudomonas, Salmonella, Serratia*, and the like. However, under such conditions, the peptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

To express hBsk in a prokaryotic cell, it is necessary to operably link the hBsk sequence to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the *int* promoter of bacteriophage λ, the *bla* promoter of the β-lactamase gene sequence of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene sequence of pBR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage λ (P_L and P_R), the *trp, recA, lacZ, lacI*, and *gal* promoters of *E. coli*, the α-amylase (Ulmanen *et al., J. Bacteriol. 162*:176-182

(1985)) and the ç-28-specific promoters of *B. subtilis* (Gilman *et al., Gene sequence 32*:11-20 (1984)), the promoters of the bacteriophages of *Bacillus* (Gryczan, In: *The Molecular Biology of the Bacilli*, Academic Press, Inc., NY (1982)), and *Streptomyces* promoters (Ward *et al., Mol. Gen. Genet. 203*:468-478 (1986)). Prokaryotic promoters are reviewed by Glick (*J. Ind. Microbiol. 1*:277-282 (1987)); Cenatiempo (*Biochimie 68*:505-516 (1986)); and Gottesman (*Ann. Rev. Genet. 18*:415-442 (1984)).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold *et al.* (*Ann. Rev. Microbiol. 35*:365-404 (1981)).

The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene. As used herein, "cell", "cell line", and "cell culture" may be used interchangeably and all such designations include progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant progeny have the same functionality as that of the originally transformed cell.

Host cells which may be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the hBsk peptide of interest. Suitable hosts may often include eukaryotic cells.

Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, mammalian cells either *in vivo*, or in tissue culture. Mammalian cells which may be useful as hosts include HeLa cells, cells of fibroblast origin such as VERO or CHO-K1, or cells of lymphoid origin and their derivatives.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences.

Another preferred host is an insect cell, for example *Drosophila* larvae. Using insect cells as hosts, the *Drosophila* alcohol dehydrogenase promoter can be used. Rubin, *Science 240*:1453-1459 (1988).. Alternatively, baculovirus vectors can be engineered to express large amounts of hBsk in insects cells (Jasny, *Science 238*:1653 (1987); Miller *et al.*, In: *Genetic Engineering* (1986), Setlow, J.K., *et al.*, eds., *Plenum*, Vol. 8, pp. 277-297).

Different host cells have characteristic and specific mechanisms for the translational and post-translational processing and modification (*e.g.*, glycosylation, cleavage) of proteins. Appropriate cell lines or host systems can be chosen to ensure the desired modification and processing of the foreign protein expressed. For example, expression in a bacterial system can be used to produce an unglycosylated core protein product. Expression in yeast will produce a glycosylated product. Expression in mammalian cells can be used to ensure "native" glycosylation of the heterologous hBsk protein. Furthermore, different vector/host expression systems may effect processing reactions such as proteolytic cleavages to different extents.

Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes are produced in large quantities when yeast are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals.

Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications. A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy

number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes peptides bearing leader sequences (i.e., pre-peptides). For a mammalian host, several possible vector systems are available for the expression of hBsk.

A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products, such as actin, collagen, myosin, and the like, may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by varying the temperature, expression can be repressed or initiated, or are subject to chemical (such as metabolite) regulation.

As discussed above, expression of hBsk in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis. Preferred eukaryotic promoters include, for example, the promoter of the mouse metallothionein I gene sequence (Hamer *et al.*, *J. Mol. Appl. Gen. 1*:273-288 (1982)); the TK promoter of Herpes virus (McKnight, *Cell 31*:355-365 (1982)); the SV40 early promoter (Benoist *et al., Nature (London) 290*:304-310 (1981)); the yeast *gal4* gene sequence promoter (Johnston *et al., Proc. Natl. Acad. Sci. (USA) 79*:6971-6975 (1982); Silver *et al., Proc. Natl. Acad. Sci. (USA) 81*:5951-5955 (1984)).

As is widely known, translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is

preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes hBsk does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as the hBsk coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as the hBsk coding sequence).

An hBsk nucleic acid molecule and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a non-replicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular molecule. Since such molecules are incapable of autonomous replication, the expression of the gene may occur through the transient expression of the introduced sequence. Alternatively, permanent expression may occur through the integration of the introduced DNA sequence into the host chromosome.

In one embodiment, a vector is employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, *Molec. Cell. Biol. 3*:280 (1983).

In a preferred embodiment, the introduced nucleic acid molecule will be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species. Preferred prokaryotic vectors include plasmids such as those capable of replication in *E. coli* (such as, for example, pBR322, ColE1, pSC101, pACYC 184, πVX. Such plasmids are, for example, disclosed by Sambrook (cf. *Molecular Cloning: A Laboratory Manual*, second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989)). *Bacillus* plasmids include pC194, pC221, pT127, and the like. Such plasmids are disclosed by Gryczan (In: *The Molecular Biology of the Bacilli*, Academic Press, NY (1982), pp. 307-329). Suitable *Streptomyces* plasmids include pIJ101 (Kendall *et al.*, *J. Bacteriol. 169*:4177-4183 (1987)), and streptomyces bacteriophages such as φC31 (Chater *et al.*, In: *Sixth International Symposium on Actinomycetales Biology*, Akademiai Kaido, Budapest, Hungary (1986), pp. 45-54). Pseudomonas plasmids are reviewed by John *et al. (Rev. Infect. Dis. 8*:693-704 (1986)), and Izaki (*Jpn. J. Bacteriol. 33*:729-742 (1978)).

Preferred eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known in the art (Botstein *et al.*, *Miami Wntr. Symp. 19*:265-274 (1982); Broach, In: *The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, *Cell 28*:203-204 (1982); Bollon *et al.*, *J. Clin. Hematol. Oncol. 10*:39-48 (1980); Maniatis, In: *Cell*

Biology: A Comprehensive Treatise, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608 (1980)).

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, i.e., transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the production of hBsk. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like).

VII. An Antibody Having Binding Affinity to an hBsk Polypeptide and a Hybridoma Containing the Antibody

In another embodiment, the present invention relates to an antibody having binding affinity specifically to a hBsk polypeptide as described above. Those which bind selectively to hBsk would be chosen for use in methods which could include, but should not be limited to, the analysis of altered hBsk expression in tissue containing hBsk.

The hBsk proteins of the present invention can be used in a variety of procedures and methods, such as for the generation of antibodies, for use in identifying pharmaceutical compositions, and for studying DNA/protein interaction.

The hBsk peptide of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide would be generated as described herein and used as an immunogen.

The antibodies of the present invention include monoclonal and polyclonal antibodies, as well as fragments of these antibodies. The invention further includes single chain antibodies. Antibody fragments which contain the idiotype of the molecule can be generated by known techniques. For example, such fragments include but are not limited to: the $F(ab')_2$ fragment; the Fab' fragments, Fab fragments, and Fv fragments.

Of special interest to the present invention are antibodies to hBsk which are produced in humans, or are "humanized" (i.e. non-immunogenic in a human) by recombinant or other technology. Humanized antibodies may be produced, for example by replacing an immunogenic portion of an antibody with a corresponding, but non-immunogenic portion (i.e. chimeric antibodies) (Robinson, R.R. *et al.*, International Patent Publication PCT/US86/02269; Akira, K. *et al.*, European Patent Application 184,187; Taniguchi, M., European Patent Application 171,496; Morrison, S.L. *et al.*, European Patent Application 173,494; Neuberger, M.S. et al., PCT Application WO 86/01533; Cabilly, S. *et al.*, European Patent Application 125,023; Better, M. *et al.*, *Science 240*:1041-1043 (1988); Liu, A.Y. *et al.*, *Proc. Natl. Acad. Sci. USA 84*:3439-3443 (1987); Liu, A.Y. *et al.*, *J. Immunol. 139*:3521-3526 (1987); Sun, L.K. *et al.*, *Proc. Natl. Acad. Sci. USA 84*:214-218 (1987); Nishimura, Y. *et al.*, *Canc. Res. 47*:999-1005 (1987); Wood, C.R. *et al.*, *Nature 314*:446-449 (1985)); Shaw *et al.*, *J. Natl.Cancer Inst. 80*:1553-1559 (1988). General reviews of "humanized" chimeric antibodies are provided by Morrison, S.L. (*Science, 229*:1202-1207 (1985)) and by Oi, V.T. *et al.*, *BioTechniques 4*:214 (1986)). Suitable "humanized" antibodies can be alternatively produced by CDR or CEA substitution (Jones, P.T. *et al.*, *Nature 321*:552-525 (1986); Verhoeyan *et al.*, *Science 239*:1534 (1988); Beidler, C.B. *et al.*, *J. Immunol. 141*:4053-4060 (1988)).

In another embodiment, the present invention relates to a hybridoma which produces the above-described monoclonal antibody. A hybridoma

is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "*Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*," Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth *et al., J. Immunol. Methods 35*:1-21 (1980)).

Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or interperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal which is immunized, the antigenicity of the polypeptide and the site of injection.

The polypeptide may be modified or administered in an adjuvant in order to increase the peptide antigenicity. Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or β-galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz *et al., Exp. Cell Res. 175*:109-124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell,

Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology, supra (1984)).

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

In another embodiment of the present invention, the above-described antibodies are detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such as horse radish peroxidase, alkaline phosphatase, and the like) fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, see (Sternberger *et al., J. Histochem. Cytochem. 18*:315 (1970); Bayer *et al., Meth. Enzym. 62*:308 (1979); Engval *et al., Immunol. 109*:129 (1972); Goding, *J. Immunol. Meth. 13*:215 (1976)). The labeled antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues which express a specific peptide.

In another embodiment of the present invention the above-described antibodies are immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, "*Handbook of Experimental Immunology*" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby *et al., Meth. Enzym. 34* Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays as well as in immunochromotography.

Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and kits disclosed

above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, "Application of Synthetic Peptides: Antisense Peptides", In *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY, pp. 289-307 (1992), and Kaspczak *et al.*, *Biochemistry* 28:9230-8 (1989).

Anti-peptide peptides can be generated in one of two fashions. First, the anti-peptide peptides can be generated by replacing the basic amino acid residues found in the hBsk peptide sequence with acidic residues, while maintaining hydrophobic and uncharged polar groups. For example, lysine, arginine, and/or histidine residues are replaced with aspartic acid or glutamic acid and glutamic acid residues are replaced by lysine, arginine or histidine.

VIII. A Method of Detecting an hBsk Polypeptide in a Sample

In another embodiment, the present invention relates to a method of detecting a hBsk polypeptide in a sample, comprising: a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present invention and assaying whether the antibody binds to the test sample. Altered levels of hBsk in a sample as compared to normal levels may indicate a specific disease.

Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available immunological assay formats (such as

radioimmunoassays, enzyme-linked immunosorbent assays, diffusion based Ouchterlony, or rocket immunofluorescent assays) can readily be adapted to employ the antibodies of the present invention. Examples of such assays can be found in Chard, *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock *et al.*, *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, *Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is capable with the system utilized.

IX. A Diagnostic Kit Comprising Antibodies to hBsk

In another embodiment of the present invention, a kit is provided which contains all the necessary reagents to carry out the previously described methods of detection. The kit may comprise: i) a first container means containing an above-described antibody, and ii) second container means containing a conjugate comprising a binding partner of the antibody and a label. In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound antibodies. Examples of detection reagents include, but are not limited to,

labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. The compartmentalized kit may be as described above for nucleic acid probe kits.

One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

X. Diagnostic Screening and Treatment

The diagnostic and screening methods of the invention are especially useful for a patient suspected of being at risk for developing a disease associated with an altered expression level of hBsk based on family history, or a patient in which it is desired to diagnose an hBsk-related disease.

According to the invention, presymptomatic screening of an individual in need of such screening is now possible using DNA encoding the hBsk protein of the invention. The screening method of the invention allows a presymptomatic diagnosis, including prenatal diagnosis, of the presence of a missing or aberrant hBsk gene in individuals, and thus an opinion concerning the likelihood that such individual would develop or has developed an hBsk-associated disease. This is especially valuable for the identification of carriers of altered or missing hBsk genes, for example, from individuals with a family history of a hBsk-associated disease. Early diagnosis is also desired to maximize appropriate timely intervention.

In one preferred embodiment of the method of screening, a tissue sample would be taken from such individual, and screened for (1) the presence of the "normal" hBsk gene; (2) the presence of hBsk mRNA and/or (3) the presence of hBsk protein. The normal human gene can be characterized based upon, for example, detection of restriction digestion

patterns in "normal" versus the patient's DNA, including RFLP analysis, using DNA probes prepared against the hBsk sequence (or a functional fragment thereof) taught in the invention. Similarly, hBsk mRNA can be characterized and compared to normal hBsk mRNA (a) levels and/or (b) size as found in a human population not at risk of developing hBsk-associated disease using similar probes. Lastly, hBsk protein can be (a) detected and/or (b) quantitated using a biological assay for hBsk activity or using an immunological assay and hBsk antibodies. When assaying hBsk protein, the immunological assay is preferred for its speed. An (1) aberrant hBsk DNA size pattern, and/or (2) aberrant hBsk mRNA sizes or levels and/or (3) aberrant hBsk protein levels would indicate that the patient is at risk for developing an hBsk-associated disease.

The screening and diagnostic methods of the invention do not require that the entire hBsk DNA coding sequence be used for the probe. Rather, it is only necessary to use a fragment or length of nucleic acid that is sufficient to detect the presence of the hBsk gene in a DNA preparation from a normal or affected individual, the absence of such gene, or an altered physical property of such gene (such as a change in electrophoretic migration pattern).

Prenatal diagnosis can be performed when desired, using any known method to obtain fetal cells, including amniocentesis, chorionic villous sampling (CVS), and fetoscopy. Prenatal chromosome analysis can be used to determine if the portion of the chromosome possessing the normal hBsk gene is present in a heterozygous state.

In the method of treating an hBsk-associated disease in a patient in need of such treatment, functional hBsk DNA can be provided to the cells of such patient in a manner and amount that permits the expression of the hBsk protein provided by such gene, for a time and in a quantity sufficient to treat such patient. Many vector systems are known in the art to provide such delivery to human patients in need of a gene or protein missing from

the cell. For example, retrovirus systems can be used, especially modified retrovirus systems and especially herpes simplex virus systems. Such methods are provided for, in, for example, the teachings of Breakefield, X.A. *et al., The New Biologist 3*:203-218 (1991); Huang, Q. *et al., Experimental Neurology 115*:303-316 (1992), WO93/03743 and WO90/09441. Delivery of a DNA sequence encoding a functional hBsk protein will effectively replace the missing or mutated hBsk gene of the invention.

In an alternative embodiment stem cell populations for either neuronal or glial cells can be genetically engineered to express a functional hBsk receptor. Such cells recombinantly expressing the hBsk receptor, can be transplanted to the diseased or injured region of the mammal's limbic system (*Neural Transplantation. A Practical Approach*, Donnet & Djorklund, eds., Oxford University Press, New York, NY (1992)). In yet another alternative embodiment, embryonic tissue or fetal neurons can be genetically engineered to express functional hBsk receptor and transplanted to the diseased or injured region of the mammal's limibic system. The feasibility of transplanting fetal dopamine neurons into Parkinsonian patients has recently been demonstrated. (Lindvall *et al., Archives of Neurology 46*:615-631 (1989)).

Studies of the molecular interactions between ligands and their receptors showed that only the extracellular domain of the receptor is involved in the special physical interaction between the molecules (Riedel *et al., Nature 324*:68-70 (1986); Riedel *et al.. EMBP J. 8*:2943-2945 (1989)). Thus, the extracellular domain of a receptor can be used as a probe to screen an expression cDNA library for the hBsk ligand or ligands. In one approach for detection of the receptor probe, placental alkaline phosphatase will be fused to the extracellular domain of a receptor, and positive clones will be detected by the presence of alkaline phosphatase activity.

An alternative approach is to isolate the putative hBsk ligand is to utilize the findings that co-expression of a receptor and its ligand in the same cells results in uncontrolled proliferation and malignant transformation (Klein *et al.*, *Cell 66*:395-403 (1991); Gazit *et al.*, *Cell 39*:89-97 (1984)). A eukaryotic cDNA expression library can be transfected into cells expressing a receptor, and the presence of a ligand will create an autocrine loop, resulting in a transformed phenotype. This approach has been successfully used by Miki *et al.*, *Science 251*:72-75 (1991), to isolate the receptor of the keratinocyte growth factor (KGF) using cells expressing KGF. In yet another alternative approach the hBsk receptor protein can be expressed in a cell line or in *Xenopus* oocytes by the recombinant techniques described above and its ligand stimulated activation of tyrosine kinase activity, as detected by an anti-phosphotryosine antibody (UBI, Happauge, New York) can be used to assay and purify the ligand. For example, cells expressing the recombinant hBsk receptor can be exposed to mammalian brain extract. The brain extracts can be fractionated by chromatography and used to assay for the presence of the ligand activity. Once an activity is identified in a particular fraction, it can be further purified by conventional biochemical techniques.

In another alternative approach, the hBsk extracellular domain can be used to screen a random peptide library (Cull *et al.*, *Proc. Natl. Acad. Sci. USA 89*:1865-1869 (1982); Lam *et al.*, *Nature 354*:82-84 (1991)). Peptides isolated can be assayed for their ligand activity in the above-described assays for drug screening.

In another embodiment of this invention, the hBsk ligand is expressed as a recombinant gene in a cell, so that the cells may be transplanted into a mammal, preferably a human in need of gene therapy. To provide gene therapy to an individual, a genetic sequence which encodes for all or part of the hBsk ligand is inserted into a vector and introduced into a host cell. Examples of diseases that may be suitable for gene therapy

include, but are not limited to, neurodegenerative diseases or disorders, Alzheimer's, schizophrenia, epilepsy, neoplasms and cancer. Examples of vectors that may be used in gene therapy include, but are not limited to, defective retroviral, adenoviral, or other viral vectors (Mulligan, R.C., *Science 260*:926-932 (1993)). The means by which the vector carrying the gene may be introduced into the cell include but is not limited to, microinjection, electroporation, transduction, or transfection using DEAE-Dextran, lipofection, calcium phosphate or other procedures known to one skilled in the art (*Molecular Cloning, A Laboratory Manual*, Sambrook *et al.*, eds., Cold Spring Harbor Press, Plainview, New York (1989)).

In another embodiment, the present invention relates to a method of administering hBsk to an animal (preferably, a mammal (specifically, a human)) in an amount sufficient to effect an altered level of hBsk in said animal. The administered hBsk could specifically effect hBsk associated functions. Further, since hBsk is expressed in brain tissue, administration of hBsk could be used to alter hBsk levels in the brain.

One skilled in the art will appreciate that the amounts to be administered for any particular treatment protocol can readily be determined. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counter indications, if any, and other such variables, to be adjusted by the individual physician. Dosage can vary from .001 mg/kg to 50 mg/kg of hBsk, in one or more administrations daily, for one or several days. hBsk can be administered parenterally by injection or by gradual perfusion over time. It can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-

aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like. See, generally, *Remington's Pharmaceutical Science*, 16th Ed., Mack Eds. (1980).

In another embodiment, the present invention relates to a pharmaceutical composition comprising hBsk in an amount sufficient to alter hBsk associated activity, and a pharmaceutically acceptable diluent, carrier, or excipient. Appropriate concentrations and dosage unit sizes can be readily determined by one skilled in the art as described above (See, for example, *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980) and WO 91/19008).

The present invention is described in further detail in the following non-limiting examples.

Example 1
Isolation of hBsk

A λ ZAP library made from mRNA isolated from the brain of a 18-week-old female human fetus was used to isolate hBsk. hBsk was isolated using a 4.3 kb mouse Bsk probe labeled with [^{32}P] by random priming. 10^6 phage plaques were lifted onto nitrocellulose filters and prehybridized and hybridized under non-stringent conditions (5X SSPE, 10X Denhardt's solution, 100 μg/ml freshly denatured salmon sperm DNA, 37% freshly deionized formamide, 2% SDS). Hybridization was performed

at 42°C for 16 hrs after prehybridization in the same buffer without the labeled probe for 2 hrs. After hybridization, the filters were washed under increasingly stringent conditions: 2X SSC, 0.5% SDS, 30 min, 50°C, 2 times; 0.1X SSC, 0.1% SDS, 50°C, 30 min, 2 times; 0.1X SSC, 0.1% SDS, 65°C. Filters were exposed to X-ray films to assess the extent of signal loss after each wash. Positive plaques were picked and purified to homogeneity. cDNA inserts in the purified positive clones were then excised from the phage vector into plasmid DNA using M13 helper phage. Partial sequence analysis (for general techniques *See*, *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989) was then performed to identify hBsk clones (See Figure 2).

Thirty-four independent potential hBsk cDNA clones were isolated from mRNA purified from a human fetal brain using a mouse Bsk probe under stringent washing conditions. Partial sequence of these demonstrate that five of these clones represent different parts of the hBs as shown schematically in Figure 1.

Clones 6-1 and 8-1 contain an entire hBsk gene and were deposited at the ATCC.

A clone containing the entire hBsk gene is constructed by 1) identifying a restriction site shared by Clones 6-1 and 8-1 which is only present in one location within these clones, 2) digesting the clones with the corresponding restriction enzyme and a restriction enzyme(s) which cuts at the 5' end of the 8-1 clone and the 3' end of the 6-1 clone, and 3) linking the fragments together with, for example, ligase. If convenient restriction enzyme sites are not present within the clones, they may be inserted into the clones using site specific mutagenesis (cf. *Molecular Cloning: A Laboratory Manual, second edition*, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

Example 2
The Biological function of hBsk

Construction of a Chimeric Receptor

To construct a M-CSFR hBsk chimeric receptor, a combination of restriction enzyme digestion and PCR approaches will be used to ligate the M-CSFR extracellular domain and the hBsk transmembrane and intracellular domains (M-CSFR/Bsk). An alternative construct, M-CSFR/hBsk 2, would contain M-CSFR extracellular and transmembrane domains and hBsk intracellular domain.

The chimeric receptor will be expressed under the LTR promoter in the pMEX expression vector (Oskam *et al.*, *Proc. Natl. Acad. Sci. USA* 85:2964-2968 (1988)) in NIH/3T3 cells to study whether it has a mitogenic effect and in PC12 cells to study whether it induces differentiation. The expression cassette pMEX-CR (CR for chimeric receptor) will be cotransfected with pSV2Neo. Neo-resistant colonies will be grown up and tested for the expression of the chimeric receptor using immunoprecipitation or Western blot analysis (Ausubel *et al.*, in *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, NY (1987)). To study if the chimeric receptor is properly localized in the cytoplasmic membrane, cell surface labeling will be performed. The labeled cells will be lysed and immunoprecipitated with either *Bsk*- or M-CSFR-specific antibodies. If the receptor is properly localized on the cell surface, a positive labeling of the chimeric product should result.

To examiner the binding property of the chimeric receptor to M-CSF, [^{125}I]-MCSF will be used to bind intact transfected or control cells. To measure the receptor binding affinity and specificity, the concentration of M-CSF needed to cause 50% inhibition of maximal [^{125}I]-MCSF binding to the cells will be determined (IC50). Scratchard analysis will be

performed on the binding competition data (Scatchard, *Ann. NY Acad. Sci. 51*:660-672 (1949)) and the dissociation constant (Kd) will be calculated. Cells transfected with vector alone will be used as controls.

To study if the chimeric receptor functions properly, the chimeric receptor will be immunoprecipitated with either M-CSFR or hBsk-specific antibodies from M-CSF-stimulated cells for various times, using unstimulated cells as a control. The precipitated receptor will be analyzed by the Western blot technique with anti-phosphotyrosine antibody to examine the activation of the receptor tyrosine kinase.

The biological effects of the stimulation of the chimeric receptor with M-CSF will be studied in NIH/3T3 cells by stimulation of DNA synthesis under low serum (calf serum at 0.3%) conditions. To determine the functions of hBsk in neuronal differentiation, the effects of the chimeric receptor activation on neurite outgrowth in PC12 cells will be studied.

[^{35}S] Methionine/Cysteine Labeling of Proteins

Cells including, but not limited to, PC12 cells, and NIH/3T3 cells, are grown to 80% confluence in 100-mm tissue culture dishes, washed twice in methionine/cysteine-free DMEM, (Dulbecco's Modified Eagle Media) starved in the same medium supplemented with 5% dialyzed fetal calf serum for 30 min, and then incubated for 2, 4, or 8 hours in the same medium with Translabel (Amersham, 70% [^{35}S] cysteine. The labeled cells will be lysed and immunoprecipitated as described below.

Cell Surface Labeling

Cells expressing the M-CSFR/hBsk chimeric receptor will be grown to 80% confluence on 175-cm^2 flasks. Cells are washed twice with PBS, removed gently with a cell scraper, and resuspended in PBS containing 6 U

of lactoperoxidase, 20 U of glucose oxidase and 2mCi of [^{125}I]. After 0, 10, and 20 min, 140 μl of 1M glucose will be added. At 30 min, the reaction is stopped by three washes in PBS. The cells are then lysed and immunoprecipitated using appropriate antibodies.

Preparation of Cell Extracts and Immunoprecipitation

Cells will be washed in Wash Buffer (HEPES 50 mM, pH 7.4, NaCl 150 mM, glycerol 10%, EDTA 10 mM, NaF 100 mM, vanadate 2 mM, $Na_4P_2O_7$ 10 mM, trypsin inhibitor 1000 U/ml, PMSF 1mM, aprotinin 1mM, leupeptin 10 μM); the cells are lysed for 30 min at 4°C in 200 μL of lysis buffer (Wash Buffer containing 1% Triton X-100) and centrifuged for 30 min at 150,000 g in a Beckman TL-100 ultracentrifuge. The extracts are then cleared twice by 15 min incubation with protein A Sepharose (40 μl of 10% gel for 200 μl of cell extracts). After a 5 min centrifugation, supernatants are mixed with appropriate antibodies adsorbed on protein-A-Sepharose and incubated for 2 h at 4°C with agitation. The samples are then centrifuged for 30 sec and the pellets are washed 6 times (3 times with wash buffer, 3 times with wash buffer supplemented with 500 mM NaCl, 0.1% Triton X-100, 0.1% SDS). The washed pellets are then resuspended in SDS-PAGE buffer and subjected to SDS-PAGE analysis. Labeled proteins are visualized by autoradiography.

Ligand Binding Study

Cells are grown in 100 mm culture dishes in DMEM to 80% confluence and then washed with PBS and incubated with 5 ml of 25 mM EDTA in PBS for 2 min. Cells are then removed from the plate, washed once with binding Buffer (100 mM HEPES, pH 7.6, 120 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 1 mM EDTA, 10 mM glucose, 15 mM sodium

acetate, 1% dialyzed BSA), and resuspended in 5 ml of Binding buffer to determine the cell number. 400 µl of this cell suspension is then incubated with [^{125}I]-M-CSF (5 pM) and increasing concentrations of unlabeled M-CFS (from 0 to 10^{-6} M) in a total volume of 500 µl for 90 min at 15°C. After incubation, cells are washed with Binding Buffer. Free [^{125}I]-MCSF is removed by six washes in Binding Buffer. Finally, the [^{125}I] radioactivity bound to the cells is determined in a λ-counter. Data obtained will be analyzed by the method of Scatchard (Scatchard, *Ann. N.Y. Acad. Sci. 51*:660-672 (1949).

Thymidine Incorporation Assay

Confluent cell monolayers in 12-well culture dishes will be grown to quiescence in medium containing 0.5% fetal bovine serum for 24 hours (h). DNA synthesis will be stimulated by adding various amount of M-CSF. Eighteen hours later, cells will be labeled for 4 h with 0.5 µCi [methyl-^{3}H] thymidine at 3 TBq/mmol, then washed three times with ice cold PBS, incubated with 1 ml of 10% trichloroacetic acid for 30 min, and washed twice with the same solution at 4°C. Cells will be then solubilized in 0.5 ml of 0.2 N NaOH, 1% SDS for 1 h at 37°C and the lysate will be brought to neutral pH with Tris buffer. The incorporated radioactivity will be determined in a liquid scintillation counter.

Construction of Recombinant Adenovirus (Ad-CR)

The recombinant adenovirus is constructed by *in vivo* homologous recombination between an adenoviral vector containing the chimeric receptor and an adenovirus deletion mutant Ad1327 genomic DNA (Stratgord-Perricaudt *et al.*, *J. Clin. Invest. 90*:626-630 (1992)) in 293 cells which express adenoviral early genes (Graham *et al.*, *J. Gen. Virol.*

36:59-72 (1977)). Briefly, 293 cells are cotransfected with 5 μg of linearized plasmid pAd-CR and 5 μg of the Large Cla I fragment (2.6-100 mu) of Ad5 DNA. After overlaying with agar and incubating for 10 days at 37°C, plaques containing recombinant adenoviruses are isolated and amplified in 293 cells, viral DNA is purified, and recombinant adenovirus plaques containing the Bsk chimeric receptor are identified by restriction cleavage and Southern analysis.

Hippocampal Neuron Cultures

Hippocampi are dissected from E18 rate embryos and collected in F10 medium (Gibco). The tissues are minced, rinsed twice with F10 medium, and the cells are dissociated by gentle trituration and collected by low speed centrifugation (500 rpm) for 30 sec. The pellet is washed again in the same medium by resuspension and centrifugation. The cell pellets are resuspended in MEM supplemented with 10% fetal calf serum, 2 mM glutamine, 25 U/ml Penicillin and 25 μg/ml) and laminin (10 μg/ml) coated 6 mm microtiter wells at a density of 70,000 cells/cm^2. Six hours following the plating of cells, the medium is changed to a serum-free medium containing 25 μg/ml insulin, 100 μg/ml transferrin, 60 μM putrescine, 20 nM progesterone, 30 nM selenium, 6 mg/ml glucose (Lu *et al.*, *Proc. Natl. Acad. Sci. USA 88*:6289-6292 (1991) and penicillin-streptomycin (25 U/ml and 25 μg/ml, respectively), and infected with the viruses at a moi of 10, 5, 2, and 1, respectively. M-CSF is added at the same time. Medium is changed every 3-4 days with the re-addition of fresh factors.

Measurement of Neurofilament Protein

Cells are fixed with 4% (v/v) paraformaldehyde for 4 hr at 4°C, permeabilized with 0.1% (v/v) Triton X-100 in PBS for 15 min, and blocked with 10% FCS in PBS for 1 h. The cells are then incubated with anti-neurofilament 20 antibody for 1 h at room temperature, washed twice with PBS containing 10% FCS, and incubated with the secondary antibody (horseradish peroxidase-conjugated) for 1 h. Following sequential washing with PBS and water, the cells are incubated with 0.2% (w/v) O-phenylenediamine and 0.02% (v/v) H_2O_2 in citrate buffer (50 mM) for 30 min. The reaction is topped by adding an equal volume of 4.5 M H_2SO_4. Product formation will be quantitated by reading the optical density of the reaction product at 492 nm.

Immunocytochemistry

Cells are rinsed twice with PBS, fixed with 4% paraformaldehyde for 30 min at room temperature, and blocked with 10% FCS in PBS containing 0.1% Triton X-100. The cells are then incubated with the primary antibodies overnight at 4°C, washed with 0.1% Triton X-100 in PBS three times and incubated with Texas Red conjugated secondary antibodies for 90 min at room temperature. The cells are washed again and positive cells are visualized under a fluorescent microscope.

Measurement of High-Affinity Uptake of GABA

High-affinity GABA uptake will be measured as described (Ip *et al.*, *J. Neuro. Sci. 11*:3124-3234 (1991)). Cells are washed in the GABA uptake buffer containing 140 mM NaCl, 2.5 mM KCl, 1mM KH_2PO_4, 1mM Na_2HPO_4, 6mg/ml glucose, 1$MgCl_2$, 1 mM $CaCl_2$, and 0.1% BSA.

Following washing, cells are incubated in the GABA uptake buffer for 5 min at 37°C. [^{3}H]-GABA is then added to a final concentration of 12 nM, and incubated at 37°C for 10 min. Cells are kept on ice and washed three times with the uptake buffer. Cells are then solubilized with 0.14 N NaOH for 2 h at room temperature, and [^{3}H]-GABA in the extract is counted. Uptake of GABA into non-neuronal cells is inhibited by the addition of 2 mM β-alanine, whereas uptake specific for neurons is verified by inhibition with nipecotic acid at 1 mM. Specific neuronal GABA uptake is determined as GABA uptake that is blocked in the presence of 1 mM nipecotic acid.

Placental Alkaline Phosphatase Activity Assay

The assay is performed by heating a portion of the supernatant at 65°C for 10 min to inactivate background phosphatase activity and then measuring the optical density at 405 nm after incubation with 1 M diethanolamine (pH 9.8), 0.5 mM MgCl, 10 μM L-homoarginine (a phosphatase inhibitor), 0.5 mg/ml BSA, and 12 mM p-nitrophenyl phosphate. The highest alkaline phosphatase-expressing clone will be selected for the purification of AP-tag-hBsk fusion protein. To concentrate and purify AP-tag-hBsk protein, the supernatant will be incubated with a monoclonal antibody to placental alkaline phosphatase coupled to CNBr-activated Sepharose. Specifically bound protein will be eluted with 144 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 50 mM sodium citrate (pH 2.5), and will be then immediately neutralized with 1 M HEPES (pH 8.0). The purified protein will be used as a probe to screen a brain expression library.

Library Screening Using AP-tag hBsk

A brain expression cDNA library will be plated at a density of 50,000 pfu per 150 mm plate. Duplicate filters will be lifted from the plates and rinsed in TBST. The filters are then blocked with TBST with 10% goat serum, rinsed once in TBST, and incubated in TBST with AP-tag-Bsk probe for 3 hours. The Filters are then washed in three changes of TBST, 3 min each. The positive clones will be detected by color formation when the filters are incubated with alkaline phosphatase substrates 5-bromo-4-chloro-3-inodyl-phosphate (BCIP, 0.017 mg/ml) and nitrobluetetrazolium (NBT, 0.33 mg/ml) in 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 5 mM $MgCl_2$. A non-specific alkaline phosphatase inhibitor, L-homoarginine (10 mM), will be added if required.

Effect of the Chimeric Receptor in Hippocampal Neurons

A vector system which is based on an adenovirus (Stratford-Perricaudet *et al.*, *J. Clin, Invest. 90*:626-630 (1992)) will be used to deliver CSFR/Bsk into hippocampal neurons from E18 rat embryos. This vector has been used successfully in the nervous system and no cytotoxicity was observed (LeGal LeSalle *et al.*, *Science 259*:988-990 (1993)). In addition, long-term expression of genes was achieved with this vector (LeGal LeSalle *et al.*, *Science 259*:988-990 (1993)).

To clone M-CSF/Bsk chimeric receptor into an adenoviral promoter, a vector plasmid, pAd-Cr, containing a chimeric receptor expressing cassette driven by M-MLV LTR promoter will be constructed. The cassette will be bordered at the 5′ end by the left end (map unit 0-1.3) of adenovirus type 5 (Ad5) and at the 3′ end by sequences from mu 9.4-17 (Bgl II-Hind II fragment of Ad5) to allow homologous recombination with

the adenoviral genome to generate recombinant virus. The resulting recombinant virus will lack the early gene E1 and therefore will be replication competent except when provided with E1 function in 293 cells (Graham *et al.*, *J. Gen Virol. 36*:59-72 (1977)).

To study the effect of hBsk chimeric receptor on the survival of hippocampal neurons, hippocampal neuron culture will be established in polyornithine- and laminin-coated plastic dishes in MEM supplemented with 10% fetal calf serum (FCS) and glutamine and infected with Ad-CR or control virus. Later, the medium will be changed to a serum-free medium containing hormone supplements. M-CSF will be added at this time. After various times of treatment, cells will be stained with antibody against neuron-specific enolase to identify neurons in the culture. The number of neurons in cultures infected with virus containing M-CSFR/hBsk or with viral vector only will be compared to determine the effect of M-CSF stimulation on the survival of specific neurons. Parallel infected cultures will be studied for hBsk protein expression at various time points using Western blot or immunoprecipitation and for ligand-dependent receptor (Kaplan *et al.*, *Nature 350*:158-160 (1991); Klein *et al.*, *Cell 65*:189-197 (1991)).

To examine if hBsk has any effect on the neurite outgrowth of the hippocampal neurons, changes in the level of neurofilament protein upon M-CSF treatment will be examined. Hippocampal neurons will be infected with virus carrying M-CSF/hBsk and treated with various concentrations of M-CSF (0.001-10 ng/ml) of M-CSF for 8 days and neurofilament protein levels will be measued by ELISA. Neurons infected with vector alone will be used as controls. To delineate which neuronal population which respond to hBsk kinase activation, the effect of M-CSF treatment of neurons expressing the chimeric receptor on the number of GABAergic and calbindin-positive neurons will be studied. Infected neurons will be treated with various concentrations of M-CSF (0.001-100 ng/ml). After 8 days of

treatment, cells will be stained with anti-GABA receptor or anti-calbindin antibodies to study the effect of hBsk activation on the survival of various neuronal populations. In addition to the immunostaining with different antibodies, the changes of the high-affinity uptake for GABA will be studied after various times of M-CSF treatment. [^{3}H]-GABA binding by the neurons in cultures infected with the virus expressing the chimeric receptor or with control virus will be compared. β-alanine will be used to inhibit the uptake of GABA into non-neuronal cells (Ip *et al.*, *Neurosci. 11*:3124-3134 (1991)).

An alternative vector system which is based on a herpes virus may also be used to deliver CSFR/hBsk into hippocampal neurons (Anderson *et al.*, *Human Gene Therapy 3*:487-499 (1992); Fink *et al.*, *Human Gene Therapy 3*:11-19 (1992)).

Example 3
Isolation of an hBsk Ligand

Screening of a cDNA Expression Library For hBsk Ligand Using an Extracellular Domain-Alkaline Phosphatase Fusion Protein As a Probe

To construct a fusion protein between the extracellular domain of hBsk and the secreted placental alkaline phosphatase (SEAP), a vector named APtag-1, constructed by Flannagan & Leder, *Cell 63*:185-194 (1990), will be used. APtag-1 contains a set of restriction sites for the insertion of the region of the hBsk cDNA encoding the extracellular domain. Downstream of the insertion sites is the full length sequence of SEAP, which will be fused to the upstream sequence.

To generate a hBsk receptor fusion protein, the 5′ end of the hBsk cDNA sequence will be inserted into APtag-1, including sequences encoding the hBsk secretion signal peptide and the entire extracellular

domain, ending immediately before the first hydrophobic amino acid of the transmembrane region. The resulting plasmid will therefore encode a fusion protein with the hBsk extracellular domain joined to a SEAP. A fusion protein will be expressed from a Moloney Murine Leukemia virus LTR promoter. The fusion construct will be transfected into NIH/3T3 cells which have been shown to express high levels of an APtag-Kit fusion protein (Flannagan & Leder, *Cell 63*:185-194 (1990)). The fusion construct will be contransfected with a selectable marker plasmid pSV2neo, and selected with G418 (400-800 μg/ml). Neo-resistant colonies will be grown in 96-well plates and screened for secretion of SEAP activity into the media (See above). The fusion protein will be concentrated, purified and used as a probe to screen a cDNA expression library from mammalian brain, preferably mouse.

Three types of positive clones are expected: (1) clones having background alkaline phosphatase activity; (2) clones which bind non-specifically to the fusion protein; and (3) clones encoding the putative hBsk ligand. Background phosphatase clones will be positive without the added probe in the presence of alkaline phosphatase substrates. To distinguish the specific from the non-specific interacting clones, extracts from bacteria expressing these clones will be used to stimulate the tyrosine kinase activity of hBsk in a hBsk expressing NIH/3T3 cells. Only the ligand will be able to stimulate activation of hBsk tyrosine activity.

It is preferable to produce the receptor probe in NIH/3T3 cells rather than bacteria to receive proper glycosylation of the hBsk extracellular domain. It has however been demonstrated that glycosylation of growth factors is often not necessary for their activity. For example, M-CSF (Metcalf, *Blood 67*:257-267 (1986) and NGF (available from Boehringer Mannheim) produced in bacteria are biologically active. Therefore, the glycosylated receptor probe should interact properly with its ligand synthesized by *E. coli* in a phase plaque during the screening.

In addition to using the Ap-tagged hBsk probe to screen for putative ligand *in vitro*, the probe can also be used in histological staining on mammalian brain section to localize expression of the ligand. Determination of the loci of expression of the *Bsk* ligand will allow for biochemical purification of the ligand from that tissue cell source further for analysis.

Functional Screening of the hBsk Ligand

An alternative approach to isolate the hBsk ligand is to utilize a functional screening approach. Full length cDNA of hBsk will be cloned into an expression vector pMEX under a MMLV LTR promoter. The hBsk expression vector will be co-transfected into NIH/3T3 cells together with pSV2Hygro containing a hygromycin β-phosphotransferase gene which confers hygromycin resistance (Gritz & Davies, *Gene 25*:179-188 (1983)). The transfected cells will be selected with hygromycin B at a concentration of 350 μg/ml. The resistant clones will be grown in 12-well plates and screened for hBsk expression with anti-hBsk antibody by Western blot analysis.

The vector system developed by Miki *et al.*, *Gene 83*:137-146 (1989), will be used to construct a directional eukaryotic cDNA library from mouse brain mRNA. The vector has a MMLV LTR promoter for the expression of cDNA inserts and a SV40 early promoter-driven Neo gene as a selectable marker. In addition, this vector contains a pBR322 replication origin, and the cDNA inserts of interest can be obtained easily by *Not I* digestion of crude Lambda DNA preparations and ligation followed by transfection of bacterial cells. The cDNA library will be constructed as described in detail by Miki *et al.*, *Gene 83*:137-146 (1989).

The cDNA library will be transfected into hBsk-expressing NIH/3T3 mouse embryo fibroblasts. Foci from transfected cells will be isolated and

tested for Neo resistance to eliminate the background transformation in NIH/3T3 cells. Genomic DNA from each Neo-resistant transformant will be cleaved by *Not I* which will release the plasmid. Digested DNA will be ligated under diluted conditions and used to transform competent bacteria. Plasmid DNA from each focus will be purified and transfected in NIH/3T3 cells with or without hBsk expression. The transformation by the putative hBsk ligand but not other oncogenes is expected to be dependent on the presence of hBsk expression. Putative clones will then be further analyzed by sequencing, the encoded protein purified and assayed for hBsk binding.

* * * * *

All publications mentioned hereinabove are hereby incorporated in their entirety by reference.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of this disclosure that various changes in form and detail can be made without departing from the true scope of the invention and appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Rutgers, The State University of New Jersey

(ii) TITLE OF INVENTION: Human Brain Specific Kinase

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: STERNE, KESSLER, GOLDSTEIN & FOX
(B) STREET: 1100 New York Ave., N.W.
(C) CITY: Washington
(D) STATE: D.C.
(E) COUNTRY: U.S.A.
(F) ZIP: 20005

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: To Be Assigned
(B) FILING DATE: 26-JUL-1995
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/279,855
(B) FILING DATE: 26-JUL-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Goldstein, Jorge A.
(B) REGISTRATION NUMBER: 29,021
(C) REFERENCE/DOCKET NUMBER: 1459.020PC00

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 202-371-2600
(B) TELEFAX: 202-371-2540

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 174 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCACCCCGGG CCCCGAGGCG CTGCTACCTG CACCTCGACG GGCTCCCCTC TGGACGTTCC 60

TTCTCCTGTG CGCCGCTACC GGACCCTCCT GGCCAGCCCC AGTAACGAAT GAATTATGAT 120

CACGCACTGT CATGGACTGA TGATCGTTCA AATGTGAGAT GATGATGATG CCTA 174

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 216 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGCATAATTT GTATCACAAA TATAAGTCTT CATCATGTGT GGTATATAGA GCATACATAG 60

AAGATATGTT ATATTCCTTA ATAAGCCTAA TTACATAATA CCTTGGATTG GTTCAATAAA 120

ATAAACTCAA AGCCATGTAA CTGAATACAA ACTGAAATTA ACAATGAATA AACATCCATT 180

AAAATAAACT TATCAATTAT TGAATAATTC TGGAGG 216

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 262 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

AGGCTTGGCT TGGCAAACGA AAACAAGATC AGAAGAGGAA AAGATGCATT TTCATAATGG 60

GCACATTAAA CTGCCAGGAG TAAGAACTAC ATTGATCCAC ATACCTATGA GGATCCCAAT 120

CAAGCTGTCC ACGAATTTGC TAAGGAGATA GAAGCATCAT GTATCACCAT TGAGAGAGTT 180

ATTGGAGCAG GTGAATTTGG TGAGTTTGTA GTGGACATTT GAACTACCAG GAAAGAGATT 240

ACCTGTGCTA TCAACCTAAG TG 262

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 223 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

ATTTTAGGAA GGCATTCTTT CCTCTTTTTT AGGGAGAGTA CTTGTTGTTT GACAGTCTCT 60

TTATTCATTC TAAAGTCATA TTAACCTTCC TTAGAAATTC TCAACCAAAT TGAGATCGAA 120

AGAAACTATG TGGCTATTTA ACTTTCCTTC TTCAATTTAC TTCAAATGAC ACCAAGATCG 180

AAGAGGCAAT AATTAGCATA TAATCTCTTG TAAGGAACAA CGC 223

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 245 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAGGTTGAAG CTTCCTCATG GGTATAACTA TGAGGTGGAC ATTTGCCGCA GCTCTGGATG 60

TGAGGTGAGG CTTTGAAGAA CCCAGGTCTG CACACTTGAC AGGTGCCATT TTTCTCTTCA 120

TATCCTGCCT TGCACATGCA TTTCCCGATG GCACCAGCAC TCCCCTTCGC TGCAGTACAT 180

TTTGGGAGGT TCATCGTCAC AGATGGTTGA CACAGAGCTG ACACTCGAGC ATGGAGATCA 240

GCTCA 245

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 176 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATAGACAGAC CACCATCATT CACGAGAGCA CTCAGCCCGC AGCTATTTCC TTCTGCCAGT 60

CTCTTTGAAC TCTGGATCTT TGCAAAAGCT CGCTGCTCTC CTGTTTTTCA TTCTCCACAT 120

TTTCTCAAGG TCCTCTTTCT TATCCTTAAG CACCTGCTTT TCTCTTTTAA AGAGTG 176

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 95 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AACACGAAGG CTGCAAAGAA CCGCACCTCC CCTAGCGGAT TTAAAAACTC TAACCGAAAA 60

AGCTGAAAGG CAAGGACAGG ACCCAGGACC TCTGA 95

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 163 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTTGATGTAT TGGTTTTCCT TGATGTTTCT CCCATTCTGA TCACTGACTC AAAGTAATAC 60

ATATTAAAGG TCCTTACAGG TCCCAGTCCT CCAGGAAGGC TGTTGCAGTC CGCAGTAAAT 120

TGAGTCTATG AGATCTGAGC ACTCATGAGA CCACGCAAGC AGA 163

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TAAACTACCA GGAAAAAGAA TTAATGTGGC TATAAAACCC TTAAAGTGGC TATACTGAAA 60

AGCGCCGG 68

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 133 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CACAACTTAC ATTACTATTC CATAACTCCA GACATCACTG GCAGAAGTAA ACTTTCGGAA 60

AGCTATGCTT CTGGGCGTCC ATCTGATTGG AATTTTCCTT GTGTGTAGCT GCTCGGACAC 120

TCAGACCGAG CAG 133

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 16, line 8.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet [X]

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
22 July 1994	ATCC 75838

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet [ ]

Plasmid, pBSK-Human clone 6-1

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
[X] This sheet was received with the international application	[ ] This sheet was received by the International Bureau on:
Authorized officer: Paul F. Urrutia, PCT International Division	Authorized officer

Form PCT/RO/134 (July 1992)

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13*bis*)

A. The indications made below relate to the microorganism referred to in the description on page 16, line 8.

B. IDENTIFICATION OF DEPOSIT — Further deposits are identified on an additional sheet ☐

Name of depositary institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit	Accession Number
22 July 1994	ATCC 75839

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* — This information is continued on an additional sheet ☐

Plasmid, pBSK-Human clone 8-1

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indications are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

For receiving Office use only	For International Bureau use only
☒ This sheet was received with the international application	☐ This sheet was received by the International Bureau on:
Authorized officer: Paul F. Urrutia, PCT International Division	Authorized officer

Form PCT/RO/134 (July 1992)

What Is Claimed Is:

1. An isolated nucleic acid molecule coding for a polypeptide comprising an amino acid sequence corresponding to human brain specific kinase, hBsk.

2. The isolated nucleic acid molecule according to claim 1, wherein the molecule has the nucleic acid sequence of the hBsk present in Clones 6-1 and 8-1 which are deposited with the ATCC.

3. The isolated nucleic acid molecule according to claim 1, wherein the molecule encodes the amino acid sequence of the hBsk present in Clones 6-1 and 8-1 which are deposited with the ATCC.

4. A substantially pure polypeptide comprising an amino acid sequence corresponding to hBsk.

5. The polypeptide according to claim 4, wherein the polypeptide comprises the amino acid sequence of the hBsk present in Clones 6-1 and 8-1 which are deposited with the ATCC.

6. A nucleic acid probe for the detection of the presence of hBsk in a DNA sample from an individual comprising a nucleic acid molecule sufficient to specifically detect under stringent hybridization conditions the presence of the molecule according to claim 1 in said sample.

7. A method of detecting hBsk nucleic acid in a sample comprising:

a) contacting said sample with the nucleic acid probe according to claim 6, under conditions such that hybridization occurs, and

b) detecting the presence of said probe bound to hBsk nucleic acid.

8. A kit for detecting the presence of hBsk nucleic acid in a sample comprising at least one container means having disposed therein the nucleic acid probe according to claim 6.

9. A recombinant nucleic acid molecule comprising, 5′ to 3′, a promoter effective to initiate transcription in a host cell and the isolated nucleic acid molecule according to claim 1.

10. A recombinant nucleic acid molecule comprising a vector and the isolated nucleic acid molecule according to claim 1.

11. A cell that contains the recombinant nucleic acid molecule according to any one of claims 9 or 10.

12. A non-human organism that contains the recombinant nucleic acid molecule according to any one of claims 9 or 10.

13. An antibody having binding affinity to the polypeptide of claim 4.

14. A method of detecting a hBsk polypeptide in a sample, comprising:

a) contacting said sample with an antibody according to claim 13, under conditions such that immunocomplexes form, and

b) detecting the presence of said antibody bound to said polypeptide.

15. A diagnostic kit comprising:

a) a first container means containing the antibody according to claim 13 and

b) second container means containing a conjugate comprising a binding partner of said monoclonal antibody and a label.

16. A hybridoma which produces the monoclonal antibody according to claim 13.

17. A bioassay for assessing candidate drugs or ligands of the hBsk receptor comprising:

a) contacting a candidate drug or ligand with a cell producing functional hBsk receptors; and

b) evaluating the biological activity mediated by said contact.

18. The bioassay of claim 17 wherein said cell is selected from the group consisting of PC12 cells, primary culture of hippocampal neurons and NIH-3T3 cells.

19. The bioassay of claim 17 wherein a source of said candidate drug or said ligand is selected from the group consisting of medium from primary cultures of hippocampal neurons, conditioned medium from PC12 cells, conditioned medium from NIH/3T3 cells and mammalian brain homogenate.

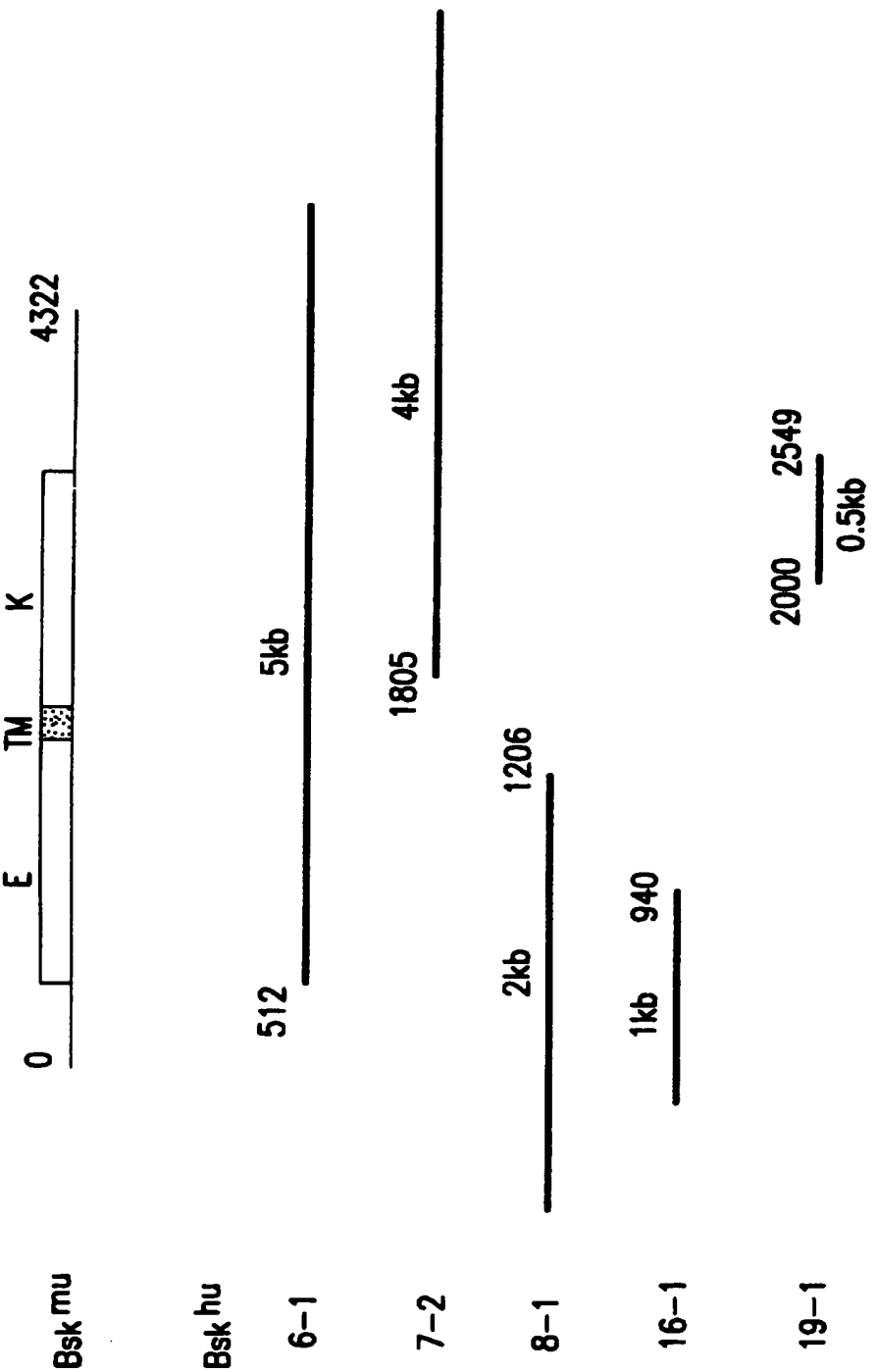
20. A method of treatment of limbic system disease in a mammal, comprising administering a therapeutically effective amount of an hBsk receptor gene in a gene delivery system to said mammal.

21. The method of claim 20, wherein said method of administering comprises microinjecting said hBsk receptor gene in said gene delivery system into said limbic system of said mammal.

22. The method of claim 20, wherein said disease is selected from the group consisting of neurodegenerative diseases, neurdegenerative disorders and neurodegenerative injuries.

23. A ligand or ligands that mediate hBsk biologic activity.

24. A method of treatment of limbic system disease in a mammal, comprising administering a therapeutically effective amount of a hBsk ligand or ligands to a mammal afflicted with limbic system disease.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8-1
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16-1
2000
2549
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19-1

FIG. 1

1 gcaccccggg ccccgaggcg ctgctacctg caactcgacg ggctcccctc

51 tggacgttcc ttctcctgtg cgccgctacc ggaccctcct ggccagcccc

101 agtaacgaat gaattatgat cacgcactgt catggactga tgatcgttca

151 aatgtgagat gatgatgatg ccta

FIGURE 2A

1 tgcataattt gtatcacaaa tataagtctt catcatgtgt ggtatataga

51 gcatacatag aagatatgtt atattcctta ataagcctaa ttacataata

101 ccttggattg gttcaataaa ataaactcaa agccatgtaa ctgaatacaa

151 actgaaatta acaatgaata aacatccatt aaaataaact tatcaattat

201 tgaataattc tggagg

FIGURE 2B

```
  1  aggcttggct tggcaaacga aaacaagatc agaagaggaa aagatgcatt
 51  ttcataatgg gcacattaaa ctgccaggag taagaactac attgatccac
101  atacctatga ggatcccaat caagctgtcc acgaatttgc taaggagata
151  gaagcatcat gtatcaccat tgagagagtt attggagcag gtgaatttgg
201  tgagtttgta gtggacattt gaactaccag gaaagagatt acctgtgcta
251  tcaacctaag tg
```

FIGURE 2C

```
  1  attttaggaa ggcattcttt cctctttttt agggagagta cttgttgttt
 51  gacagtctct ttattcattc taaagtcata ttaaccttcc ttagaaattc
101  tcaaccaaat tgagatcgaa agaaactatg tggctattta actttccttc
151  ttcaatttac ttcaaatgac accaagatcg aagaggcaat aattagcata
201  taatctcttg taaggaacaa cgc
```

FIGURE 2D

1 gaggttgaag cttcctcatg ggtataacta tgaggtggac atttgccgca

51 gctctggatg tgaggtgagg ctttgaagaa cccaggtctg cacacttgac

101 aggtgccatt tttctcttca tatcctgcct tgcacatgca tttcccgatg

151 gcaccagcac tccccttcgc tgcagtacat tttgggaggt tcatcgtcac

201 agatggttga cacagagctg acactcgagc atggagatca gctca

FIGURE 2E

1 atagacagac caccatcatt cacgagagca ctcagcccgc agctatttcc

51 ttctgccagt ctctttgaac tctggatctt tgcaaaagct cgctgctctc

101 ctgtttttca ttctccacat tttctcaagg tcctctttct tatccttaag

151 cacctgcttt tctcttttaa agagtg

FIGURE 2F

```
1   AACACGAAGG CTGCAAAGAA CCGCACCTCC CCTAGCGGAT TTAAAAACTC
51  TAACCGAAAA AGCTGAAAGG CAAGGACAGG ACCCAGGACC TCTGA
```

FIGURE 2G

```
1   TTTGATGTAT TGGTTTTCCT TGATGTTTCT CCCATTCTGA TCACTGACTC
51  AAAGTAATAC ATATTAAAGG TCCTTACAGG TCCCAGTCCT CCAGGAAGGC
101 TGTTGCAGTC CGCAGTAAAT TGAGTCTATG AGATCTGAGC ACTCATGAGA
151 CCACGCAAGC AGA
```

FIGURE 2H

1 TAAACTACCA GGAAAAAGAA TTAATGTGGC TATAAAACCC TTAAAGTGGC

51 TATACTGAAA AGCGCCGG

FIGURE 2I

1 CACAACTTAC ATTACTATTC CATAACTCCA GACATCACTG GCAGAAGTAA

51 ACTTTCGGAA AGCTATGCTT CTGGGCGTCC ATCTGATTGG AATTTTCCTT

101 GTGTGTAGCT GCTCGGACAC TCAGACCGAG CAG

FIGURE 2J

INTERNATIONAL SEARCH REPORT

l. .national application No.
PCT/US95/09334

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/ 6, 7.1, 172.3; 514/44; 530/ 350, 388.24; 536/ 23.1, 23.2, 24.31

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE
SEARCH TERMS: Zhou, Paulhiac, Sajjadi, Pasquale, brain specific kinase, bsk, cek, cek7, eph, ehk, tyrosine kinase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A, P	Trends in Biotechnology, Volume 13, issued January 1995, Martin, "Gene therapy and pharmacological treatment of inherited neurological disorders", pages 28-35, see entire document.	20-22
Y, P	Journal of Biological Chemistry, Volume 269, Number 43, issued 28 October 1994, Shao et al. "cDNA cloning and characterization of a ligand for the cek5 receptor protein tyrosine kinase", pages 26606-26609, see entire document.	1-24

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
07 SEPTEMBER 1995	12 OCT 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MICHAEL J. NEWELL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

In. .ational application No.
PCT/US95/09334

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Gene, Volume 148, issued 1994, Siever et al., "Identification of a complete cek7 receptor protein tyrosine kinase coding sequence and cDNAs of alternatively spliced transcripts", pages 219-226, see entire document.	1-24
A	Oncogene, Volume 8, issued 1993, Sajjadi et al., "Five novel avian Eph-related tyrosine kinases are differentially expressed", pages 1807-1813, see entire document.	1-24
Y	Oncogene, Volume 8, issued 1993, Maisonpierre et al., "Ehk-1 and Ehk-2: two novel members of the Eph receptor-like tyrosine kinase family with distinctive structures and neuronal expression", pages 3277-3288, see entire document.	1-24
Y	Journal of Neuroscience Research, Volume 37, issued 1994, Zhou et al., "Isolation and characterization of Bsk, a growth factor receptor-like tyrosine kinase associated with the limbic system", pages 129-143, see entire document.	1-24
Y, P	Oncogene, Volume 10, issued 1995, Fox et al., "cDNA cloning and tissue distribution of five human EPH-like receptor protein tyrosine kinases", pages 897-905, see entire document.	1-24
Y	British Journal of Cancer, Volume 69, issued 1994, Tuzi et al., "Eph, the largest known family of putative growth factor receptors", pages 417-421, see entire document.	1-24
Y, P	Brain Research and Molecular Brain Research, Volume 28, issued 1995, Schulz et al., "Isolation and expression analysis of tyro3, a murine growth factor receptor tyrosine kinase preferentially expressed in adult brain", pages 273-280, see entire document.	1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09334

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01N 43/04; A61K 31/70; C07H 21/02, 21/04; C07K 1/00, 14/00, 16/00, 17/00; C12N 15/00; C12P 21/08; C12Q 1/68

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

435/ 6, 7.1, 172.3; 514/44; 530/ 350, 388.24; 536/ 23.1, 23.2, 24.31